

# Assisted Hatching Reduces the Age-Related Decline in IVF Outcome in Women Younger than Age 43 Without Increasing Miscarriage or Monozygotic Twinning

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**Purpose:** Our purpose was to examine the benefits of assisted hatching in our program and to determine whether the procedure increases the implantation of nonviable embryos or monozygotic twinning.

**Methods:** Consecutive *in vitro* fertilization cycles using assisted hatching were compared with historical controls. The impact of assisted hatching was analyzed according to the woman's age. Outcome measures were clinical pregnancy, implantation rate, delivery rate, multiple pregnancy, spontaneous abortion, and incidence of monozygotic twins.

**Results:** The implantation rate was increased in women aged 35–39 and markedly increased in women aged 40–42. There was no change in spontaneous abortions and no increase in monozygotic twins.

**Conclusions:** Assisted hatching is a safe and highly effective adjunct to *in vitro* fertilization for women aged 35–42 and did not increase spontaneous abortion or monozygotic twinning.

**KEY WORDS:** assisted hatching; *in vitro* fertilization; monozygotic twins; spontaneous abortion.

## INTRODUCTION

Assisted hatching (AH) arose out of the observation that areas of thinning of the zona pellucida correlated better with embryo implantation than any other morphologic criterion (1). Subsequent controlled studies showed that the creation of an artificial gap in the zona using acidic Tyrode's significantly increased embryo implantation (2). The enhancement of implantation

increases with age, being more prominent after age 38, but still almost two-fold at ages 35–38 (2). AH was also shown to increase implantation with a moderate increase in the level of follicle-stimulating hormone (FSH) on day 3 of menses and in women with multiple failed *in vitro* fertilization (IVF) cycles (2,3). Despite these very promising findings, there have been relatively few reports of successful use of AH in other IVF programs (4–6). Published reports have consisted of relatively small numbers of pregnancies, many of which were "ongoing." Finally, the safety of AH has been recently questioned with the report of increased monozygotic (MZ) monoamniotic twinning with zona procedures (7).

The present study was done to evaluate the clinical pregnancy rate and delivery rate with AH to determine whether AH increases the implantation of nonviable embryos. The type and incidence of monozygotic twinning were examined and compared with those in non-AH pregnancies to determine whether our AH technique increases monozygotic twinning. We were also interested in quantitating the impact of AH on our program in general and, particularly, for women of age 35 or older.

## MATERIALS AND METHODS

We progressively incorporated AH into our program by first applying AH to women of age 39 or older in 1992–1993. During 1993 the indications were expanded to include a serum FSH level  $\geq 15$  (Lee Co Diagnostics, Inc., Southfield, MI) or 8 (Immulite; Diagnostic Products Corp., Los Angeles, CA) and couples with at least two failed IVF cycles. In 1995 we lowered the age criterion to 35 years. During late 1994 and 1995 and 1996 we performed AH in women having

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intracytoplasmic sperm injection (ICSI). Results of AH in these women who were younger than age 35 were not included, but these cycles were included in the assessment of monozygotic twinning.

Clinical pregnancy, spontaneous abortion, and delivery were defined as in the U.S. IVF Registry (8). AH results were compared with our IVF outcomes in 1990 and 1991, the 2 years immediately preceding our use of AH. Rates of spontaneous abortion with AH were compared with our rates before AH and those reported in the 1994 U.S. Registry. The incidence of MZ twinning was compared to that in non-AH pregnancies during the same period.

Our clinical and laboratory techniques have been reported previously (9,10). The only substantial change has been the adoption of the Wallace catheter for embryo transfer when AH was begun. We used mid-luteal leuprolide acetate throughout the study, with the dose reduced by one-half for poor responders.

AH was carried out on the morning of the third day following oocyte retrieval. The embryo was stabilized on a holding pipette in phosphate-buffered saline (PBS) under warm mineral oil on the heated stage of an inverted microscope (Diaphot; Nikon Inc., New York). A pipette (outer diameter, 10  $\mu\text{m}$ ; inner diameter, 7  $\mu\text{m}$ ) loaded with acidic Tyrode's was used to create an opening in the zona approximately 20  $\mu\text{m}$  in diameter at the outer zona, tapering to 15  $\mu\text{m}$  at the inner zona. The embryo was immediately moved to another part of the drop and then washed twice in 2–3 ml of PBS, then twice with Ham's F10, before being placed back into culture in modified Ham's F10.

Medrol, 16 mg per day, and tetracycline, 1.0 g per day, each in four divided doses, were given starting on the evening of oocyte retrieval for 4 days (11). Embryo transfer was done 1–4 hr after AH, using a Wallace catheter (Edwards–Wallace catheter; Marlow Technologies Inc., Willoughby, OH) and 20–30  $\mu\text{l}$  of medium.

## RESULTS

Table I shows the AH results during 1992–1993 compared with 1990–1991, prior to starting AH. The rates of implantation and pregnancy were only marginally higher during these first 46 cases.

Table II shows the AH results during 1994–1996. The implantation rate (IR) in low-prognosis women younger than age 35 (25) was increased to a level similar to the average for these young women in 1990–1991. The IR was increased from 14 to 21% in women

aged 35–39 compared with before AH in 1990–1991 ( $P < 0.005$ ,  $\chi^2$ ). In women aged 40–42, the IR increased more than two-fold, to 11 to 12%, but increased only marginally in women older than age 42. In 1994–1996, 53 of 484 embryos implanted in women older than age 40, compared with 5 of 117 in 1990–1991 ( $P < 0.05$ ). The delivery rate in women aged 35–39 increased to the level attained previously in women younger than age 35. One of four women aged 40–42 delivered viable infants. There were no deliveries in women older than age 42.

Twelve (16%) of 73 AH pregnancies in women younger than age 40 aborted. Sixteen (37%) of 43 AH pregnancies in women older than age 40 aborted. These rates compare to 17 and 33%, respectively, in our IVF program during 1989 through 1991 and 19 and 37% reported in the 1994 U.S. Registry (8).

One (0.7%) of 136 AH pregnancies resulted in a MZ twin compared with 3 (0.9%) of 320 non-AH IVF pregnancies. All MZ twins were diamniotic.

Table III shows the impact of AH on our IVF program. In women aged 35–39 the delivery rate increased to 43%, a level similar to that attained previously in women younger than age 35. The rate of all patients younger than age 40 increased from 32 to 45%. The delivery rate in women older than age 40 doubled from 10 to 20%.

## DISCUSSION

For the purpose of this study we have considered IVF couples to be divided into two populations: women younger than age 35, with a normal level of FSH and having their first or second IVF cycle, comprising a group with an excellent prognosis; and women older than age 35, with an increased FSH level or having failed at least two IVF cycles, having a reduced prognosis. Following a short learning curve, AH increased the delivery rate in low-prognosis women to the same level as in younger, good-prognosis women, resulting in more than a 10% increase in the delivery rate in the total group of women younger than age 40. In women aged 40–42, one of four had a viable delivery with AH, a rate equivalent to the average for women younger than age 40 in the U.S. IVF registry in 1994 (8). Although data are insufficient at present, women older than age 42 should be cautioned that their prognosis is poor and may not be improved by AH. Since the beginning of our IVF program we have not identified any single factor that has had as great an impact on our IVF results.

**Table I.** Rates of Clinical Pregnancy, Implantation, Delivery, and Multiple Delivery with Assisted Hatching (AH) in 1992–1993 Compared with the 2 Preceding Years Without AH

	1990–1991			1992–1993	
	<35	35–39	>40	<40	>40
Age (yr)					
Clinical pregnancies (%)	63/136 (46)	52/147 (35)	3/32 (9)	1/6 (17)	6/40 (15)
Implantation rate (%)	101/521 (19)	83/598 (14)	5/117 (4)	2/27 (7)	17/189 (9)
Delivery rate (%)	52/136 (38)	39/147 (27)	2/32 (6)	0/6 (0)	4/40 (10)
Multiple deliveries (%)	19/52 (37)	13/39 (33)	1/2 (50)	0 (0)	2/4 (50)

**Table II.** Rates of Clinical Pregnancy, Implantation, Delivery, and Multiple Delivery with Assisted Hatching in 1994–1996 by Age Group

	Age (yr)					
	<35	35–39	40	41	42	>42
Clinical pregnancies (%)	7/14 (50)	64/121 (53)	10/26 (38)	17/41 (41)	8/16 (50)	2/20 (10)
Implantation rate (%)	16/63 (25)	114/546 (21)	13/121 (11)	25/203 (12)	10/89 (11)	5/71 (7)
Delivery rate (%)	7/14 (50)	50/121 (41)	6/26 (23)	10/41 (24)	6/16 (38)	0/20 (0)
Multiple deliveries (%)	4/7 (57)	21/50 (42)	1/6 (17)	3/10 (30)	1/6 (17)	0 (0)

Our success rate also increased in women younger than age 35, due in part to the use of AH in some low-prognosis women. During this time we also performed ICSI for couples with a low strict morphology and did salpingectomy before IVF in some women with a hydrosalpinx. Because these two factors also define a low prognosis for implantation, these techniques contributed to an improved chance of pregnancy. Finally, the use of the Wallace catheter may have also led to a general increase in success.

During the first 2 years we achieved only a marginal improvement in the rate of implantation. AH is highly technique dependent. With experience it can be done rapidly, thus limiting temperature and pH changes around the oocyte, and the size and shape of the gap in the zona can be fashioned in a consistent manner. It may be advantageous to start with the full range of couples who could derive benefit, so that the technique is done frequently enough to minimize the learning curve.

Because AH increases implantation of embryos that were otherwise unable to escape from the zona, the

technique could result in implantation of poor-quality embryos destined to abort. We observed rates of spontaneous abortion with AH that were identical to our usual rates and the same as those in a much larger population, consisting of all programs reporting to the 1994 U.S. IVF Registry. Accumulation of much larger numbers will add further assurance that AH does not increase the rate of spontaneous abortion.

An increased rate of monozygotic, monoamniotic twins was reported with “zona procedures” (7) that included the use of smaller openings in the zona, which would be more prone to pinching the embryo during the hatching process. The report did not specify whether AH with acidic Tyrode’s was associated with this phenomenon. We observed the same rate of MZ twinning with AH as in our other IVF pregnancies, and none were monoamniotic. Although this is reassuring, couples should continue to be advised of this potential rare complication and it is important that the opening of the zona should always be adequate to minimize this risk.

## CONCLUSIONS

AH was observed to be a very effective and safe adjunct to IVF in certain low-prognosis couples. It is a sophisticated technique that may involve a significant learning curve before optimal results are achieved. The increased level of training for laboratory personnel and the modestly increased cost for these couples were more than offset by the individual and program improvements achieved.

**Table III.** Impact of Assisted Hatching on Delivery Rate<sup>a</sup> (%)

Age (yr)	1990–1993	1994–1995
<35	96/250 (38.4)	27/55 (49)
35–39	77/290 (26.6)	37/86 (43)
<40	173/540 (32.0)	64/141 (45.4)
40+	8/81 (10)	13/64 (20.3)

<sup>a</sup> As reported to the U.S. IVF Registry.

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